

Amendments to the Specification:

Please insert the following new paragraph at page 1, line 6 following the title:

C1 ¶ This application is a continuation application of U.S. Application Serial No. 09/240,639, now U.S. Patent 6,350,447, filed on January 29, 1999, entitled "Methods and Compositions Relating to CD-39-like Polypeptides and Nucleic Acids," Attorney Docket No. 28110/36120, which is herein incorporated by reference in its entirety. ¶

Please replace the existing paragraph beginning at page 96, line 12, with the following rewritten paragraph:

C2 --First, a novel murine family member was cloned by low stringency screening of mouse cDNA libraries with a human CD39L1 cDNA clone (Chadwick, B.P. & Frischauf A.-M., 1997, Mamm. Genome 8:668-672). A 1738 bp cDNA clone was isolated from an adult mouse testis cDNA library (Stratagene Ltd., Cambridge, UK) and sequenced. DNA sequence comparisons with the human CD39L1 cDNA sequence showed moderate DNA homology of approximately 39% identity). An open reading frame (ORF) could be detected for the cDNA sequence, but indicated that the cDNA clone did not contain the initiation methionine codon and, therefore, did not extend to the 5' end. Database searches with the mouse cDNA sequence identified two mouse EST clones that extended the cDNA sequence at the 5' end (Accession Nos. AA116990 and AA120757). The EST clones were resequenced. The cloned and the resequenced nucleotide sequences were analyzed and were combined appropriately to yield the nucleotide sequence (SEQ ID NO:7) depicted in FIG. 1, and referred to herein as mCD39L4 or mNTPase. The sequence revealed an ORF from nucleotides 205 to 1599 with the ATG at nucleotide 205 having a moderate match to the initiation start site for vertebrates (AAGAAUAUGG (SEQ ID NO: 18) for mNTPase versus GCCGCCAUGG (SEQ ID NO: 19); Kozak, M., 1989, J. Biol. Chem. 108:229-241). The derived amino acid sequence is also shown in FIG. 2 (SEQ ID NO:8). No apparent polyadenylation signal existed, although the cDNA clone isolated contained a poly-A tail. ¶

Please replace the existing paragraph beginning at page 98, line 17, with the following rewritten paragraph:

C3
The FISH study revealed the presence of mNTPase on mouse Chr. 12 at chromosome band E. To confirm the location of the mNTPase gene on mouse Chr. 12, linkage analysis was carried out upon the European Collaborative Interspecific Backcross (EUCIB). PCR primers were designed to the 3' untranslated region of the mNTPase cDNA sequence and used for PCR by use of mouse genomic DNA from the two parental mouse strains, Mus spretus and C57BL/6. A polymorphism was detected between the two strains by SSCP analysis and was used for the mapping. (PCR conditions: 48°C 20 sec., Primer 1: CCAGACTGTAAATCTTTTGG (SEQ ID NO: 20); Primer 2: AGGGAATGTAATAAGGGTAG (SEQ ID NO: 21); conditions: 94°C 2 minutes; 35 cycles of 94°C 20 sec. 72°C 20 sec., 72°C 1 min; product size: 320 bp).

Please replace the existing paragraph beginning at page 99, line 13, with the following rewritten paragraph:

C4
The nucleotide sequence of CD39 (Accession No. S73813), CD39L1 (Accession No. U91510), and mNTPase (see Section 6, above) were used in TBLASTX searches against entries in the expressed sequence tag (EST) database at EMBL/GenBank, using the Bork server through EMBL-Heidelberg (<http://www.bork.embl-heidelberg.de/>). cDNA clones for homologous IMAGE EST entries were obtained from the Human Genome Mapping Project Resource Centre (HGMP, Hinxton, UK). DNA was prepared with QiaTip-100 (Qiagen), and the cDNA was sequenced by primer walking with a fluorescence labeled dye-terminator cycle sequencing kit according to the manufacturer's instructions (PRISM Ready Dye-Deoxy Terminator Premix from Applied Biosystems Inc.) and electrophoresed on an ABI 373 (Perkin-Elmer). Overlapping EST clones were identified by searching with the nucleotide sequence against entries in the EST database using BLAST-N (<http://www.ncbi.nlm.nih.gov:80/cgi-bin/BLAST/nph-blast?form>).

Please replace the existing paragraph beginning at page 100, line 13, with the following rewritten paragraph:

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Members of the CD39-like gene family were mapped in the human genome by PCR screening of the GeneBridge-4 radiation hybrid ~~panel~~-mapping panel obtained from the HGMP

Resource Centre (Hinxton, UK) (Gyapay, G. et al., 1996, Hum. Mol. Genet. 5:339-346). PCR-positive radiation hybrid clones were organized into the GeneBridge-4 HGMP-RC subset order using the HGMP radiation hybrid mapping World Wide Web (WWW) site (<http://www.hgmp.mrc.ac.uk/cgi-bin/contig/contig/rhmapper.pl>) and mapping data for each gene were obtained from the Whitehead server (<http://www.genome.wi.mit.edu/cgi-bin/rhmapper.pl>). The chromosomal location for each gene was confirmed by PCR screening of the monochromosomal hybrids obtained from the HGMP Resource Centre. PCR primers were designed for the 3' untranslated region (UTR) of each gene and titrated for a unique human-specific PCR product. PCR conditions: CD39L2, Primer 1, 5'-CTGCTTGAGTGACGTCTCTG-3' (SEQ ID NO: 22); Primer 2, 5'-CACATGAGGTTCAGCTCGTG-3' (SEQ ID NO: 23); 94°C for 2 min; 38 cycles of 94°C for 20 s, 54°C for 20 s, 72°C for 20 s; 72°C for 2 min. Product size is 362 bp). CD39L3, Primer 1: 5'-GTGAAGTGGCTGCCTTCAGG-3' (SEQ ID NO: 24); Primer 2, 5'-CCTTTGACTCGGGACTCCAG-3' (SEQ ID NO: 25); 94°C for 2 min; 38 cycles of 94°C for 20 s, 56°C for 20 s, 72°C for 2 min. Product size is 281 bp). CD39L4. Primer 1, 5'-GAACTGCTGCCTAACCCTC-3' (SEQ ID NO: 26); Primer 2, 5'-ATTGATGGGTCTTGGGATTGC-3' (SEQ ID NO: 27); 94°C 2 for min; 38 cycles of 94°C for 20 s, 56°C for 20 s; 72°C for 20 s; 72°C for 2 min. Product size is 234 bp. PCR products were analyzed by electrophoresis through 3.5% NuSieve agarose gels (Flowgen).

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cont

Please replace the existing paragraph beginning at page 101, line 7, with the following rewritten paragraph:

Identification of partial human CD39L2 sequence was described in the Example presented in Section 6, above. The CD39L2 insert was used to isolate additional clones from a human adult breast epithelial cDNA library (ZR75), a human T-leukemia cell line J6 cDNA library (Jurkat), and a human keratinocyte stem cell cDNA library (KER). Of 23 cDNA clones that were isolated and sequenced, all but one appeared to be alternatively spliced or unspliced. Within the 2762 bp cDNA that appeared to be neither unspliced or alternatively spliced, an ORF extending to nucleotide 1600 containing ACRs I-IV was identified. Two ATG codons with a poor match to the consensus translation initiation site were found at nucleotide positions 148 and 232 (AUGUGAAUGA (SEQ ID NO: 28) at 148 and ACAAGGAUGA (SEQ ID NO: 29) at 232

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versus consensus GCCGCCAUGG (SEQ ID NO: 19); Kozak, M., 1989, J. Biol. Chem. 108:229-241). Based on homology to mNTPase, the ATG at nucleotide position 232 is the initiation codon. (See FIG. 9 for a depiction of the CD39L2 amino acid sequence that results from translation from the upstream, position 148, start codon; such a form of CD39L2 as well as nucleotide sequences that encode this form of the polypeptide are also intended to be included as part of the present invention.) A single polyadenylation signal of AAUAAA (SEQ ID NO: 30) was identified at nucleotide position 2700, 22 nucleotides 5' of the poly(A) tail of the human CD39L2 cDNA.A

Please replace the existing paragraph beginning at page 102, line 27, with the following rewritten paragraph:

C1

An additional CD39-like gene and polypeptide sequence, referred to herein as CD39L4, was also identified. A TBLASTX search of the NCBI EST database with the full cDNA sequence for the mNTPase was performed. A human EST clone was sequenced, and an ORF was identified extending to nucleotide 529 of 2260 nucleotides that contained ACR I only and an ATG codon at position 256. In the same reading frame, downstream of the stop codon at nucleotide 529, an ORF extending to nucleotide 1792 contained ACRs II, III, and IV. Further analysis of the nucleotide sequence revealed a putative intron with splice donor and acceptor sites that conform to the 5' gt...3' ag rule (Breathnach and Chambon, 1981, Ann Rev. Biochem. 50:349-383; splice donor CAGgtcacttatggagcctg (SEQ ID NO: 31) at nucleotide position 470, acceptor ccatggacaaaatagGAC (SEQ ID NO: 32) at position 710, exon sequence underlined). Further analysis of the sequence revealed that removal of the 251-bp putative intron would result in a contiguous ORF containing ACRs I-IV. The hypothesis that this sequence does indeed constitute an intron was only confirmed by isolation and sequencing of three additional cDNA clones (CD39LAJ1-3) from the Jurkat library, one of which contained the 251 bp.¶

Please replace the existing paragraph beginning at line 1 of the Abstract, with the following rewritten paragraph:

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¶The present invention provides novel polynucleotides and proteins encoded by such polynucleotides, along with therapeutic, diagnostic and research utilities for these

Chadwick, et al.

U.S. Application No. 09/905,589

Chadwick
polynucleotides and proteins. In particular, the polypeptides and polynucleotides of the
invention comprise amino acid and nucleic acid sequences of novel CD39-like gene and gene
products.
